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CHAPTER

4

Hampered influenza-specific IgG B cell responses whereas IgM and IgA responses are maintained in monoclonal gammopathy of undetermined significance

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ABSTRACT

Introduction

Patients with monoclonal gammopathies frequently have deficient immune function that is condition-related. The degree of this immune deficiency increases with progression of disease. As a result, patients with monoclonal gammopathies are prone to infections that include influenza virus. Monoclonal gammopathy of undetermined significance (MGUS) patients with high monoclonal protein elicit poor IgG responses to influenza vaccination.

Methods

We therefore investigated whether the poor influenza-specific IgG response observed in MGUS translated to poor seroprotection rates in this cohort. Furthermore, we analysed the memory B cell subsets in MGUS as well as quantifying the influenza-specific memory B cells after administration of trivalent inactivated influenza vaccine. Peripheral memory B cell subsets were measured by flow cytometry and stimulated total and influenza-specific memory B cells were detected using an enzyme-linked immunospot (ELISpot) assay at day 0 as well as at day 7- and day 28 post-vaccination.

Results

MGUS show low seroprotection rates (37%) with the Hemagglutination inhibition (HI) response correlating with the lower H1N1-specific IgG titers. The frequency of IgG+ cells in the IgD-CD27+ fraction at day 0 correlated with the fold increase in HI titers as well as the H1N1-specific IgG titers at day 28 post-vaccination in HC suggesting a recall memory response. In contrast, the frequency of the IgG+ cells in the IgD-CD27+ fraction is decreased at baseline in MGUS. Furthermore, the frequency of influenza-specific IgG memory B cells is significantly lower post-vaccination in MGUS in comparison to HC, as measured by ELISpot.

Despite the poor IgG response, MGUS elicit significant IgM and IgA antibody responses to influenza vaccination. However, the protective capacity of these two antibodies is questionable in MGUS.

Conclusion

MGUS is associated with poor IgG memory response that is likely to impair the long maintenance of serologic response.

INTRODUCTION

As a precursor condition to multiple myeloma (MM), MGUS does not present with clinical features of MM or other lymphoproliferative malignancies [1]. However, it is characterized by monoclonal immunoglobulin <30g/L, normal to decreased levels of IgG as well as by B lymphocyte percentages ranging from low to normal [2-4]. Additionally, there is increased susceptibility to infections in MGUS [5]. Furthermore, immune responses to influenza vaccination vary with M-protein levels as MGUS with high M-protein (>15g/L) has lower influenza-specific IgG titers which they fail to expand post-vaccination [6].

Influenza vaccination-induced immunity is primarily antibody based, relying on the activation of naïve B cells as well as the recall of memory B cells (MBCs) to rapidly respond upon re-encounter with antigen. Clonally expanded B cells can differentiate into MBCs or plasma cells that secrete antibodies at an increased rate as well as with higher affinity for antigen [7]. Several subsets of MBCs have been identified based on the expression of IgD and CD27: IgD⁺CD27⁺ classical memory switched (IgG⁺ or IgA⁺) cells, CD27⁺ unswitched memory cells that predominantly express IgD and IgM, but also IgM-only cells. Furthermore, IgD⁺CD27⁻ (double negative (DN)) memory B cells have been described. Although a definite origin of the different memory B cell subsets remains to be established, it is proposed that isotype switched cells originate from germinal center (GC) reactions while IgM memory may be early emigrants from the GC which began participating in the GC reaction but exited before isotype switching [8]. Alternatively, IgM memory may develop through GC-independent pathways [9]. On the other hand, CD27⁺IgD⁺IgM⁺ cells may represent a recirculating fraction of marginal zone B cells [10][9]. Mutated, isotype switched DN B cells contain fewer somatic hypermutations in their immunoglobulin genes and have increased IgG3 use, further distinguishing them from their CD27⁺ counterparts [11].

Characteristics of the B cell response, and predominantly the nature of long-term B cell memory, are key determining factors of the protective capacity of many vaccines. These MBCs are of importance when the protective efficacy of a vaccine is largely determined by its ability to elicit a humoral response as in influenza vaccination. Indeed, parenteral vaccination with the seasonal trivalent inactivated vaccine induces systemic immune response on which protection is based. The influenza-specific circulating antibodies induced by vaccination decline within 6 months while longer-lasting MBCs pool remains to respond upon antigen re-encounter, in so doing heightening resistance to infection [12][13].

There is limited data concerning the B cell response to influenza vaccination in MGUS. We evaluated the peak effector responses by measuring antigen-specific titers by both ELISA and hemagglutination inhibition test. Furthermore, we investigated whether the inferior response to influenza vaccination in high monoclonal protein MGUS was associated with alteration in total as well as antigen-specific MBC responses to influenza.

METHODS

Study population

Patients were included in the study when they fulfilled the standard diagnostic criteria for MGUS[14]. For control purposes, sex and age matched healthy individuals were included parallel to the MGUS patients. Healthy controls with monoclonal protein in their serum were excluded. The patient characteristics have been previously described [6]. All participants included gave written informed consent in accordance with the Declaration of Helsinki. The institutional medical ethics committee of University Medical Centre Groningen approved the study. All MGUS patients and controls received an intramuscular injection of the influenza vaccine (Influvac, Solvay Pharmaceuticals, Netherlands) between October 2010 and January 2011. The vaccine, a subunit preparation of licensed 2010-2011 trivalent inactivated virus, contained A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008.

Cell and serum isolation

Venous blood samples were obtained at the day of vaccination (day 0), 7 days and 28 days post-vaccination. PBMCs were isolated using CPT vacutainer tubes according to manufacturer's instructions. The PBMCs were frozen in RPMI 1640 (Roswell Park Memorial Institute medium; Lonza, Verviers, Belgium), supplemented with 10% human pooled serum, 50 µg/mL gentamicin (Gibco, Paisley, UK) and 10% dimethylsulfoxide (Merck, Germany) and then stored in liquid nitrogen until further analysis. Serum was separated by centrifugation and stored at -20°C until further analysis

Influenza-specific IgA and IgM antibodies by ELISA

The level of anti-influenza specific serum IgA and IgM antibodies against A/H1N1 and A/H3N2 were determined by an in-house enzyme-linked immunosorbent assay (ELISA). ELISA plates (Costar) were coated with 1 µg/mL of A/H1N1 and A/H3N2. The plates were incubated overnight on a shaker. Subsequently, serum samples collected at day 0, day 7 and day 28 following vaccination were added to the plates and then serially diluted (1:4) in PBS /0.05% Tween-20 /2% BSA. To detect influenza-specific antibodies, 1µg/ml goat anti-human IgA-HRP (Southern Biotech) or 1µg/ml mouse anti-human IgM-HRP (Southern Biotech) conjugate antibodies were used followed by incubation with 3,3',5,5'- tetramethylbenzidine (TMB) substrate. The substrate reaction was stopped by adding H₂SO₄ (UMCG pharmacy). Absorbance at 450nm was read with an Emax microplate reader and antibody concentrations calculated by SOFTmax PRO software (Molecular Devices, Sunnyvale, USA). For a standard curve, a dilution series of human IgG standard, N protein (Siemens) was used on every plate and the antibody contents of the samples were read from the linear part of the sigmoid curve.

Hemagglutination Inhibition assay

Hemagglutination Inhibition (HI) test to A/California7/2009/H1N1 was performed according to standard techniques as previously described [15]. The HI assay was performed using guinea pig erythrocytes and egg-grown influenza viruses as antigens.

Memory B cell assay

PBMCs were plated in 24-well plates at a cell concentration of 0.5×10^6 cells/ml was in RPMI 1640/10%FCS supplemented with $0.1 \mu\text{g/ml}$ IL-21 (Immunotools, Germany), $0.1 \mu\text{g/ml}$ B cell activating factor (BAFF) (PeproTech, USA) and $3.2 \mu\text{g/ml}$ CpG-ODN-2006 (InvivoGen). For negative control PBMCs were cultured in medium (RPMI 1640, supplemented with $50 \mu\text{g/mL}$ gentamicin and 10% FCS) alone. Cells were subsequently incubated for 6 days at 37°C , 5% CO_2 .

The samples were run in duplicates for detection of cells secreting total IgG antibodies as well as cells secreting TIV-specific IgG. MAIPSWU10 multiscreen filtration plates (Millipore, USA) were pre-wetted with 70% ethanol for 2 min and washed 3 times with $150 \mu\text{L/well}$ PBS (phosphate buffered saline; UMCG pharmacy). For coating, TIV (Influvac, Solvay Pharmaceuticals, The Netherlands) was diluted to $1 \mu\text{g/ml}$ in PBS. To detect total Ig secreting cells, wells were coated with $2.5 \mu\text{g/ml}$ goat anti-human IgG (Bethyl Laboratories, USA). Wells with PBS only were used as uncoated controls. The plates were incubated overnight at 4°C . After washing the plates 3 times with $150 \mu\text{L/well}$ 0.01% Tween-20 (Sigma-Aldrich, USA), plates were blocked with 2% BSA and incubated at 37°C for 2 hours. The cultured PBMC were washed thoroughly before counting. The cells were subsequently resuspended in RPMI 1640 (+ $50 \mu\text{g/mL}$ gentamicin)/10% FCS and added to the plates in duplicates with $200 \mu\text{L}$ cell suspension/well. The plates were incubated at 37°C for 18 hours. After the incubation, cells were discarded and the plates were washed with PBS /0.01% Tween-20 ($6 \times 150 \mu\text{L/well}$). Subsequently, $100 \mu\text{L/well}$ anti-human IgG-HRP (Bethyl Laboratories, USA) was added and plates incubated at room temperature for 1 hour. Unbound conjugate antibody was then washed away with PBS ($3 \times 150 \mu\text{L/well}$) and then developed using TMB substrate (Sanquin, NL) in the dark, at room temperature. To stop the reaction, the plates were washed extensively under the tap and dried in a light-shielded place. Finally, the number of spots per well were quantified with an ELISpot plate reader (A.EL.VIS, Germany).

Immunofluorescent staining and flow cytometric analysis of B cell subsets

PBMCs were counted by the Coulter Counter (Beckman Coulter, USA) and a cell suspension of 2×10^6 cells/mL was made. The following monoclonal antibodies were used in this study: CD19 (e-fluor 605), CD24 (PerCP- E-Fluor 710), CD27 (APC-E-Fluor 780), CD38 (PE-Cy7), all obtained from eBioscience. IgG (FITC) from Santa Cruz Biotechnology, IgD (V450) and IgM (APC) were from BD Biosciences. Cells were incubated with directly conjugated monoclonal antibodies for 60 minutes at 4°C .

After staining, cells were washed with 1% BSA (Sigma-Aldrich, USA). Nine color flow cytometric analysis was carried out on the BD LSR II (BD Biosciences, USA) with BD FACSDiva Software. Cells were gated on SSC versus FSC to collect lymphocytes and then on CD19 cells versus SSC to gate B cells. Between 0.5×10^5 and 1×10^6 cells were collected. Further analysis for B cell subsets was done by Kaluza (Beckman Coulter, USA). We measured the frequency of CD19+: IgM memory (IgD+/ IgM+/ CD27+), switched memory B cells (IgD-/ CD27+/CD38low /CD24+) as well as CD27- memory (IgD-/ CD27-) and plasmablasts (IgD-/ CD27+/CD38 high/CD24-).

Statistical analysis

Data analysis was done by Graphpad Prism 5 (Graphpad software). Data are presented as median (range) if not otherwise stated. The Mann–Whitney test was used to compare groups. The Spearman rank test was used to test correlations between variables. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Reduced Hemagglutination Inhibition response in MGUS

We analysed the serum Hemagglutination Inhibition titers before and 28 days after vaccination to evaluate whether the antibody production to one of the seasonal vaccine strains mirrors the responses observed by ELISA analysis. H1N1-specific IgG responses were established in the same MGUS cohort as for this study and were recently reported separately [6]. Hemagglutination inhibition titers were recorded according to criteria used to assess the immunogenicity of influenza vaccines [16]: i.e. seroprotection rates (percentage of vaccinees who achieved titers of $\geq 1:40$ post-vaccination), seroconversion (the proportion of subjects with a 4 fold or greater rise in antibody titer from the prevaccination titer), Geometric mean titers (GMT) of H1N1 before and post-vaccination and mean fold increase (MFI) in GMT. The inferior response of MGUS patients observed by ELISA was also paralleled by HI antibody titer response. Correlations between the HI titer and serum IgG levels were observed at day 28 post-vaccination for healthy controls (Spearman's $r = 0.72$; $p = 0.008$) as well as for MGUS (Spearman's $r = 0.58$; $p = 0.008$) (Figure 1A).

No difference in HI antibody titers between controls ($n=19$) and MGUS ($n=19$) were detected before vaccination. In both groups, titers of influenza specific antibodies increased after vaccination, but responses were dampened in MGUS ($p=0.07$). The fold increase in GMT was lower in MGUS (1.3) compared to healthy controls (2.6) (Figure 1B). The poor increase in antibody titers therefore resulted in lower seroconversion rates, with only 3 (16%) MGUS patients seroconverting compared to 42% of healthy controls. The seroprotection rates achieved in healthy controls (58%) were higher than those achieved in MGUS (42%), however without reaching significance ($p=0.06$).

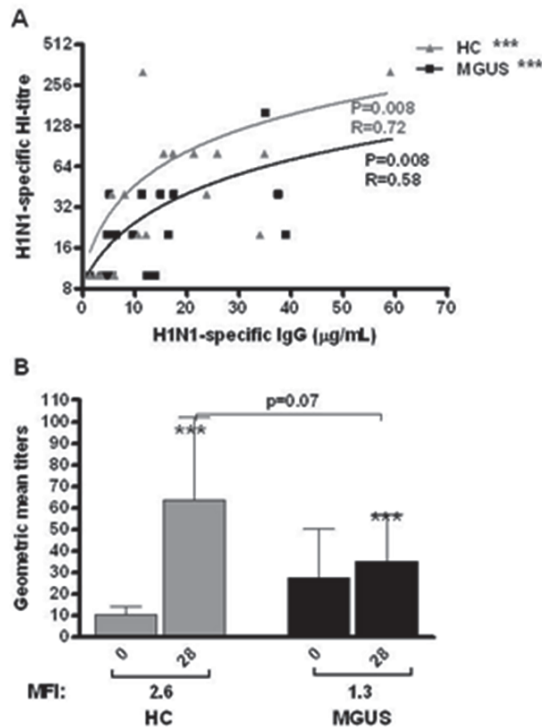


Figure 1. Hemagglutination inhibition antibody responses of HC and MGUS to H1N1 component of 2010 TIV. (A) Correlation curve for H1N1 HI titer and H1N1-specific IgG in HC (grey triangles) and MGUS (black squares) at day 28. (B) Geometric mean titers against H1N1 (A/California/7/2009) at day 0 and day 28 post-vaccination for HC (n=19) and MGUS (n=19). Bars and error bars denote group median and interquartile range. Mean fold increase in GMT is shown as numerical values below. ** $p<0.01$; *** $p<0.001$.

The frequency of TIV-specific IgG memory B cells does not increase following vaccination in MGUS

We have analysed the composition of the peripheral B cell pool in 5 HC and 6 MGUS patients in which additional PBMCs were available. To further explore influenza vaccination-induced alterations in the B cell compartment, we compared circulating B cell subsets at baseline to those obtained post-vaccination (day 7 and day 28). No statistically significant difference in the relative frequencies of CD27+ memory B cells and plasmablasts was observed between HC and MGUS at baseline (data not shown). Following vaccination, the frequencies of the B cell subsets remained relatively constant in HC except for plasmablasts; which showed a trend to an increase (but not statistically significant) at day 7 post-vaccination (data not shown).

Additionally, to investigate immunoglobulin expression on B cells, we evaluated the expression of IgG on IgD⁺CD27⁺ B cells. At baseline, the majority of the cells were of IgG isotype for both HC and MGUS. However, the frequency of these IgG⁺ cells was significantly lower in MGUS compared with HCs ($p=0.05$) (Figure 2A). The lower frequency of IgG⁺ cells in the IgD-CD27⁺ fraction in MGUS at baseline was due to a concomitant increase in IgM⁺ cells in the IgD-CD27⁺ fraction ($p=0.0037$).

Furthermore, The frequency of these IgG⁺ cells in the CD27⁺ B fraction demonstrated positive associations with the H1N1-specific serum IgG at day 28 in HCs (Spearman's $r=0.7033$, $p=0.0073$) and MGUS (spearman's $r=0.7494$, $p=0.002$). Following vaccination (day 7), there was a significant decrease in the frequency of IgG⁺ IgD- CD27⁺ cells in HC ($p=0.04$), however the decrease was not significant in MGUS. Additionally, the frequency of IgG⁺ in the isotype-switched CD27⁺ fraction at day 0 was positively correlated with the fold increase in hemagglutination inhibition titers at day 28 in HCs only (Spearman's $r=0.5522$, $p=0.0328$) (Figure 2B).

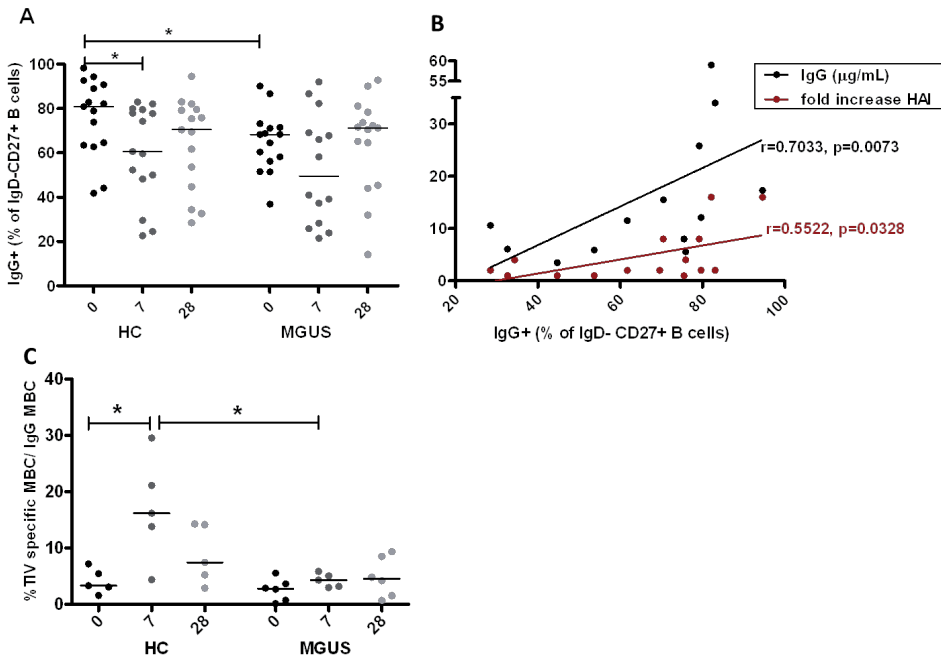


Figure 2. Analysis of influenza vaccination-induced changes in IgG responses. (A) The changes in frequencies of IgG⁺ IgD- CD27⁺ cells in response to influenza vaccine challenge in HC and MGUS at day 0, day 7 and day 28. (B) The frequency of the IgG⁺ (CD27⁺ cells) correlates with IgG titers and the fold increase in HAI in HC. (C) IgG secreting TIV-specific memory B cells in HC and MGUS at day 0, day 7 and day 28 post-vaccination. The number of TIV-specific memory B cells was calculated as a % of total IgG secreting MBCs. * $p < 0.05$

Therefore, the increase in influenza-specific IgG titers is associated with higher numbers of total IgG⁺CD27⁺ cells.

Influenza-specific memory B cell levels are expressed as the frequency of total IgG-switched memory B cells (total IgG secreting cells) to normalize the data for differences in cell recovery as well as for changes in the overall memory B cell population. In order to detect the effect of influenza vaccination, we assayed the number of influenza-specific IgG MBCs at day 7- and day 28 post-vaccination. There were detectable TIV-specific IgG memory B cells at baseline for both HC (range 12 to 74 per 10⁵PBMCs) and MGUS (range 0 to 106 per 10⁵PBMCs). However, TIV-specific IgG memory B cells were absent in 1 MGUS patient at baseline. The TIV-specific IgG memory B cells averaged 4.1% (1.6- 7.2) of total IgG memory B cells in HC and 2.6% (0- 5.6) in MGUS. Vaccination resulted in a significant increase in the frequency of TIV-specific IgG memory B cells in HC ($p=0.03$) but not in MGUS at day 7 post-vaccination. This resulted in HC having significantly higher percentages of TIV-specific IgG memory B cells ($p=0.03$) that averaged 17% of total IgG memory B cells in comparison to 4.3% in MGUS where the frequencies of the TIV-specific IgG memory B cells did not show a significant increase (Figure 2C). Of note is that the number of total IgG memory B cells at day 28 post-vaccination negatively correlate with monoclonal protein concentration (Spearman's $r = -0.87$, $p=0.03$) (data not shown).

MGUS patients mount significant IgA and IgM response to influenza vaccination

Baseline frequencies of DN (IgD-CD27-) memory B cells as well as IgM+IgD+CD27+ memory B cells were comparable between HC and MGUS. However, vaccination resulted in an increase in the frequency of DN memory B cells at day 7 in MGUS ($p=0.01$) (Figure 3A). Furthermore, IgM+ IgD+ CD27+ memory B cells were decreased in MGUS in response to vaccination at day 7 ($p=0.01$) (Figure 3B). This decrease at day 7 post-vaccination may indicate a difference in responding cells in comparison to HCs as the frequency of these cells is lower than those in HC at the same time-point ($p=0.009$).

We further examined humoral immune responses in 19 HC and 19 MGUS patients. We determined the H1N1 influenza-specific IgM and IgA antibody responses before vaccination (day 0) as well as after vaccination (day 7 and day 28 post-vaccination). Influenza-specific IgM and IgA were detected at baseline in both HCs and MGUS. Vaccination resulted in a significant increase in both IgM ($p=0.0009$) and IgA ($p=0.005$) levels at day 7 post-vaccination in HCs with the levels being maintained at day 28 post-vaccination. Similarly, MGUS showed an increase in H1N1 influenza-specific IgM ($p=0.002$) (Figure 3C) and IgA ($p=0.0003$) (Figure 3D) antibody titers at day 7. However, MGUS had a trend to higher day 7 IgM titers in comparison to HCs, though not statistically significant ($p=0.07$).

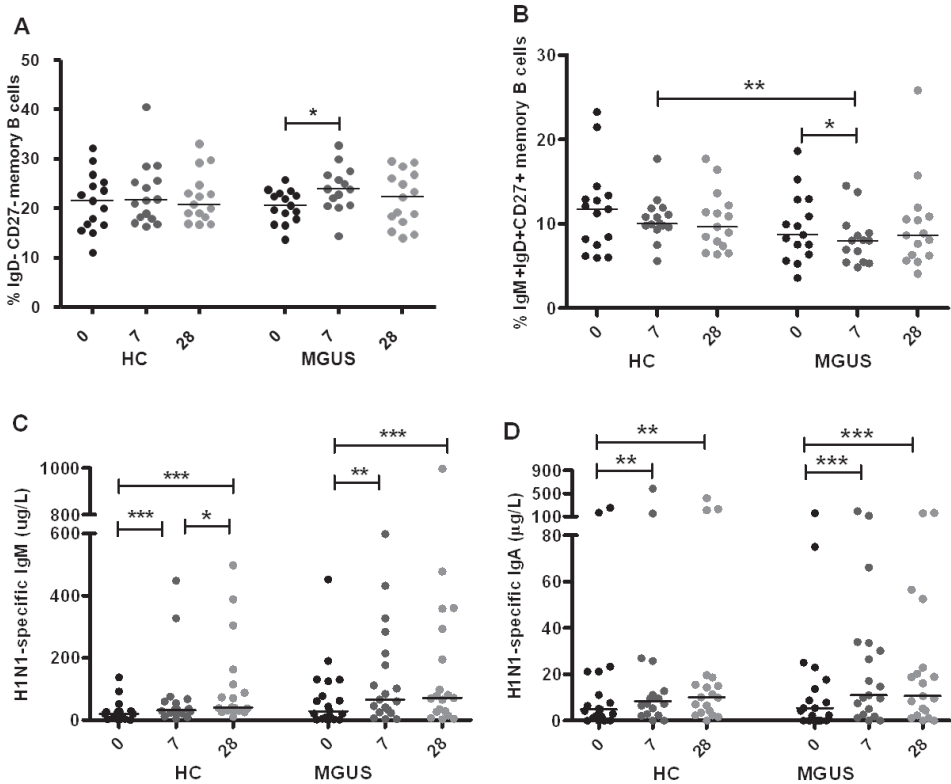


Figure 3. Effect of influenza vaccination upon peripheral B cell subset proportions and antibody titers. Frequencies of (A) IgD⁺ CD27⁺ memory B cells and (B) IgM⁺ IgD⁺ CD27⁺ memory B cells at day 0, day 7- and day 28 post-vaccination. Vaccination results in a significant increase in H1N1-specific IgM (C) and as well as IgA (D) in both HC and MGUS. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

DISCUSSION

Serum titers of hemagglutination inhibiting antibodies are an universally established correlate of protection for influenza with seroprotection rate accepted as a proxy end point [17, 18]. The HI results in our study confirmed hampered vaccine-specific antibody responses as observed by IgG ELISA as we showed that MGUS had reduced HI response. Moreover, H1N1 specific IgG antibody levels correlate with the HI response[6]. The poor post-vaccination influenza specific IgG antibody levels observed in MGUS may therefore predict or mirror response to influenza vaccination. The seroprotection rate was lower in MGUS (37%) in comparison to age matched HCs (58%) suggesting that these MGUS patients are likely to be less protected by vaccination.

After TIV vaccination, naïve and memory B cells that encounter antigen differentiate into effector B cells that appear in the circulation and peak around

day 7 and subsequently decline [19]. In this study, we see a non-significant increase in total plasmablasts at day 7 post-vaccination in both HC and MGUS. However, we did not selectively look at influenza-specific antibody secreting cells to determine the association between the serum response and effector cells. We observed TIV specific MBCs in both HC and MGUS before vaccination. This is in agreement with other studies where pre-existing influenza specific MBCs are present in the circulation [20] and reflect prior vaccination or natural infection. Previous reports have shown that following polyclonal stimulation and ELISpot, antigen-specific IgG MBCs account for up to 6% of the total IgG memory at day 30 post-vaccination [19, 20]. The responses we observed in this study were higher with TIV-specific MBCs making up to 29% of total IgG MBCs at day 7 and up to 14% at day 28 post-vaccination in HCs. The differences in the immune responses observed could be due to the differences in the composition of the TIV vaccines in the various years. Furthermore, all the HC and MGUS patients in this study were vaccinated the previous year with A/California/2009 that was present in the current TIV. Therefore, a recall response was likely to be elicited. Another difference is that the previous studies used *Staphylococcus aureus* Cowan, CpG and Pokeweed mitogen [19, 21] as polyclonal stimuli while we used IL-21, BAFF and CpG. There may therefore be differences in the MBC activation efficiency between the assays. However this discrepancy was accounted for by measuring the frequencies of TIV-specific MBC in the activated total MBC population rather than as numbers of TIV-specific MBC per stimulated PBMCs.

The expansion of MBCs following vaccination is enriched for antigen-specific cells in HC. However, vaccination did not result in a significant increase in antigen-specific MBCs in MGUS. This poor antigen-specific memory response is likely to impair the maintenance of serologic response [22]. Notably, MGUS had lower frequencies of IgG+ IgD- CD27+ cells at baseline and influenza-specific IgG secreting cells are significantly lower in response to vaccination. Moreover, the higher levels of IgG+IgD-CD27+ cells in HC at baseline were associated with higher fold-increase in the HI titers but not for MGUS implying that higher frequency of IgG+IgD-CD27+ cells are advantageous for antibody responses. Interestingly, we noted that in a subset of MGUS assayed by ELISpot, high monoclonal protein concentration is associated with lower (total) IgG MBCs following vaccination. This implies that, at least in this small subset of MGUS, high monoclonal protein concentration is associated with a limited humoral immunologic memory that will potentially limit the response to influenza vaccination.

In the IgD- CD27+ fraction, there is a decrease in the frequency of IgG+ cells that is compensated by an increase in IgM+ cells in MGUS at day 0 before vaccination. This may then limit the repertoire of IgG+ cells that is available to respond to vaccination. While, there is evidence of hampered IgG responses in MGUS, the IgM response is not negatively affected. Indeed, serum H1N1-specific IgM titers

in MGUS were comparable to HC as they also increased following vaccination. Furthermore, the change in frequencies in IgM+ (IgD+CD27+) memory B cells frequencies at day 7 may reflect differences in responding cells between HCs and MGUS. Some IgM memory B cells may differentiate into cross-reactive IgM antibody secreting cells leading to the increase in H1N1-specific IgM as observed. The rest of the IgM memory may be recruited into the secondary response to compensate for the hampered IgG response.

Interestingly, DN memory B cells are also substantially expanded in the peripheral blood of MGUS at day 7, suggesting their participation in the early phase of memory responses although their antigen specificity was not determined. Whether distinct pathways are responsible for the induction of CD27- and CD27+ memory B cells after antigen challenge remain debatable. However, these CD27- memory B cells may result from incomplete germinal center reactions therefore explaining their failure to acquire CD27 [23, 24]. This also explains their lower somatic hypermutation rates in comparison to CD27+ counterparts [25, 26]. A study by Moir and colleagues showed that influenza-specific IgG MBCs can be found in the CD27- compartment albeit at frequencies 10 times lower than in the CD27+ compartment of HC [25]. This would suggest that the influenza-specific response within the CD27-IgG+ B-cell subset would not be as effective as from its CD27+ counterpart.

We analyzed the relationship between memory response to influenza vaccination as well as the peripheral blood B cell subsets as they are easily accessible. However, by measuring peripheral blood B-cell subsets instead of antigen-specific B cells we indirectly assess the cells involved in the response to vaccination. Another limitation was the small group sizes of the HC and MGUS included in the different analyses. Nevertheless, we found several differences between the HC and MGUS but larger sample sizes are needed to confirm our results.

In conclusion, we have shown that MGUS elicit a significant IgM and IgA antibody responses to influenza vaccination despite the restricted IgG B cell response. Poor antigen-specific memory response in MGUS is likely to impair the maintenance of serologic response. The poor IgG memory response is related to high monoclonal protein in MGUS suggesting defect in B cell immunity.

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